bio-protocol Preprint

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crRNA selection

FK Francois Kroll JR Jason Rihel

Updated date: Mar 5, 2021

An abbreviated version of this protocol was published in eLIFE in Jan 2021

A simple and effective F0 knockout method for rapid screening of behaviour and other complex phenotypes

DOI: 10.7554/eLife.59683

Detailed protocol

Hi Benan –
Sorry, I'm just seeing your question here.
I think we then discussed this by email; but so it is public:

1- I typically order the 2 nmol crRNA quantity, but naturally it depends on how many times you think you will use it. To give you an idea, 2 nmol are re-suspended in 10 uL Duplex buffer, and you use 1 uL to make RNPs, so you should be able to make ~ 7 times (to acount for dead volume) fresh RNPs with it. Once resuspended, I would aliquot the crRNA or tracrRNA, so you avoid freeze-thaw cycles. I try to take each aliquot out of the freezer less than 3 times (not that I have tested this). I keep my crRNA or tracrRNA vials at -80C.
2- Perfectly fine to prepare the RNPs on injection day. I find it more convenient to prepare them the day before. In that case, I keep them overnight in the 4C fridge on some ice, so it is just above freezing temperature. IDT posted some useful data & recommendations on storage conditions: https://eu.idtdna.com/pages/education/decoded/article/stability-of-crispr-reagents-under-different-freezing-storage-and-thawing-conditions . When pooling the RNPs at the end of the protocol, you obtain ~ 6 uL. Based on IDT's data, I have also started dividing this in two aliquots. I keep the first one in the fridge overnight to inject the next day; and I place the other one in the -80C freezer. If I need to repeat the experiment, I simply use the one I kept at -80C. Each aliquot is ~ 3 uL, and I load 0.9 uL in the needle when I inject, so this gives me at least one other shot if ever I break the needle or else. Disclaimer 1: I have not done sequencing on embryos I have injected with the -80C aliquot yet, I am trusting IDT's data here. Disclaimer 2: I think Figure 3 in IDT's post linked above is freeze-thaw cycles of the Cas9 alone, I would remain careful about freeze-thaw cycles of anything RNA, tracrRNA or crRNA or gRNA or assembled RNP.

A protocol is available on protocols.io: dx.doi.org/10.17504/protocols.io.bfgyjjxw. I hope it is detailed enough. Feel free to send any feedback, I will happily improve it.
Do let me know if anything unclear or you need any precisions

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Kroll, F. and Rihel, J. (2021). crRNA selection. Bio-protocol Preprint. bio-protocol.org/prep909.
2. Kroll, F., Powell, G. T., Ghosh, M., Gestri, G., Antinucci, P., Hearn, T. J., Tunbak, H., Lim, S., Dennis, H. W., Fernandez, J. M., Whitmore, D., Dreosti, E., Wilson, S. W., Hoffman, E. J. and Rihel, J.(2021). A simple and effective F0 knockout method for rapid screening of behaviour and other complex phenotypes. eLIFE. DOI: 10.7554/eLife.59683

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